Amino Acid Racemisation Dating of a Last Interglacial Estuarine Deposit at Largs, New South Wales

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A Late Pleistocene age of 103 ± 15.5 ka BP is assigned to an estuarine shell bed at Largs in the lower Hunter Valley, New South Wales. The numeric age is based on the extent of leucine racemisation in the arcoid bivalve *Anadara trapezia* and a model of apparent parabolic racemisation kinetics. Although characterized by contrasting racemisation rates within and between genera, the relative extent of racemisation for the amino acids alanine, aspartic acid, glutamic acid, lysine, phenylalanine and proline are in accord with values previously reported for fossil molluscs from last interglacial coastal deposits in southern Australia. The amino acid racemisation data reported here provide a firmer basis with which to correlate the Largs estuarine deposit to the last interglacial maximum (ca. 134 to 118 ka BP; substage 5e of the marine $\delta^{18}O$ record).

INTRODUCTION

Since the early observations of Abelson (1954, 1956) that protein residues may remain in fossils for considerable intervals of time, an extensive literature has emerged on the application of time-dependent amino acid racemisation reactions to Quaternary dating (Miller and Brigham-Grette 1989; Murray-Wallace 1993; Wehmiller 1993). Earlier reviews of the method are provided by numerous workers (Kvenvolden 1975; Schroeder and Bada 1976; Williams and Smith 1977; Davies and Treloar 1977; Wehmiller 1984). In this paper we apply the amino acid racemisation method to assess the age of an estuarine shell bed at Largs, near the town of Maitland in the lower Hunter Valley, New South Wales.

In the protein of living organisms, amino acids are bound in peptides as left-handed molecules (L-amino acid: *laevorotatory*), a phenomenon that has been related to enzymic reactions (Williams and Smith 1977). With death, the enzymic reactions that formerly maintained the disequilibrium condition cease (i.e. exclusively L-amino acids) and amino acids then slowly and progressively interconvert from a left-handed to a right-handed counterpart (D-amino acid: *dextrorotatory*). This process is termed amino acid racemisation. The interconversion of L- to D-amino acids continues until an equilibrium mixture is attained (i.e. D/L = 1). Depending on the nature of the materials and the diagenetic temperature history, this process can take several hundred thousand years.

The Largs deposit was first described by David and Etheridge in 1890 and in view of the importance of this site for reconstructions of the Late Quaternary environmental history of eastern Australia, has been the subject of other investigations (Iredale 1951;

Murray-Wallace et al. 1988; Thom and Murray-Wallace 1988; Leary 1992). Host to a diverse assemblage of intertidal to shallow subtidal molluscs, the deposit provides a rare opportunity in eastern Australia to quantify sea-level during the last interglacial maximum (substage 5*e* of the marine oxygen isotope record). Despite earlier attempts (Murray-Wallace et al. 1988; Thom and Murray-Wallace 1988), reliable numeric ages for this deposit have remained elusive. This work presents the results of amino acid racemisation analyses for a wider range of mollusc genera than previously reported from this site (Murray-Wallace et al. 1988), as well as a numeric age for the deposit based on a model of apparent parabolic racemisation kinetics (Mitterer and Kriausakul 1989; Murray-Wallace and Kimber 1993). The age assigned to the Largs deposit based on the extent of leucine racemisation, represents the first numeric age assigned to a Late Quaternary deposit in eastern Australia, using the amino acid racemisation method.

METHODS

A trench 4 m in length and 1.5 m deep was excavated in the embankment of a Late Pleistocene river terrace of the Hunter River, near the town of Largs (Grid Reference 693805 Maitland Sheet 1:25 000; Fig. 1). The surfaces of the pit were cleaned with a brush and samples were collected for analysis of the molluscan fossil assemblage and for amino acid racemisation and radiocarbon dating. Sediment samples were also collected for description using a binocular microscope. X-ray diffraction analysis was undertaken to determine aragonite-calcite content of molluscs and to assess sample integrity for dating. Amino acid racemisation analyses followed established methods (Kimber and Griffin 1987; Murray-Wallace 1993) and were undertaken for the total acid hydrolysate for several amino acids. Measurement of the amino acid residues was performed on a Hewlett-Packard 5890A gas chromatograph using a 25m coiled, fused silica capillary column with the stationary phase Chirasil-L-Val. Analyses were generally performed on the hinge region of bivalve molluscs using 1g of shell calcium carbonate. The present day mean annual temperature for the site, a relevant consideration for amino acid racemisation studies, is 17.9°C (Australian Climatic Averages).

THE LARGE SHELL BED

Stratigraphy and sedimentology

A shallow excavation on the footslope of a large embankment revealed four lithologically-distinct units, as previously noted for this site (Iredale 1951; Thom and Murray-Wallace 1988; Figs. 2 and 3). A summary of the stratigraphic and sedimentologic characteristics of the deposit is given here. The basal unit (unit A) consists of 2 m of light grey, fine- to very fine-grained well sorted muddy sand, as revealed through augering in the floor of the pit. Binocular microscope analysis revealed a subangular grain morphology dominated by quartz and lithic fragments. A gradational contact separates unit A from an overlying unit (unit B) of well-sorted fine-grained, mottled quartzose sands that is 1.2 m thick. Whole shells and shell fragments do not occur within this unit. The main shell bed (unit C) overlies mottled quartzose sands, and contains a diverse assemblage of mollusc genera. The unit is of variable thickness ranging between 50 to 65 cm. The sediments comprise well-sorted, predominantly fine-grained, yellowish brown quartz sands. Both articulated and disarticulated bivalve molluscs are common, with the assemblage dominated by the estuarine species *Anadara trapezia*. Calcium carbonate accounts for 60% of the total sediment on a dry weight basis. Thin section analysis revealed that the non-carbonate fraction consisted of 40% quartz, 30% lithic fragments, 25% clay and 5% heavy
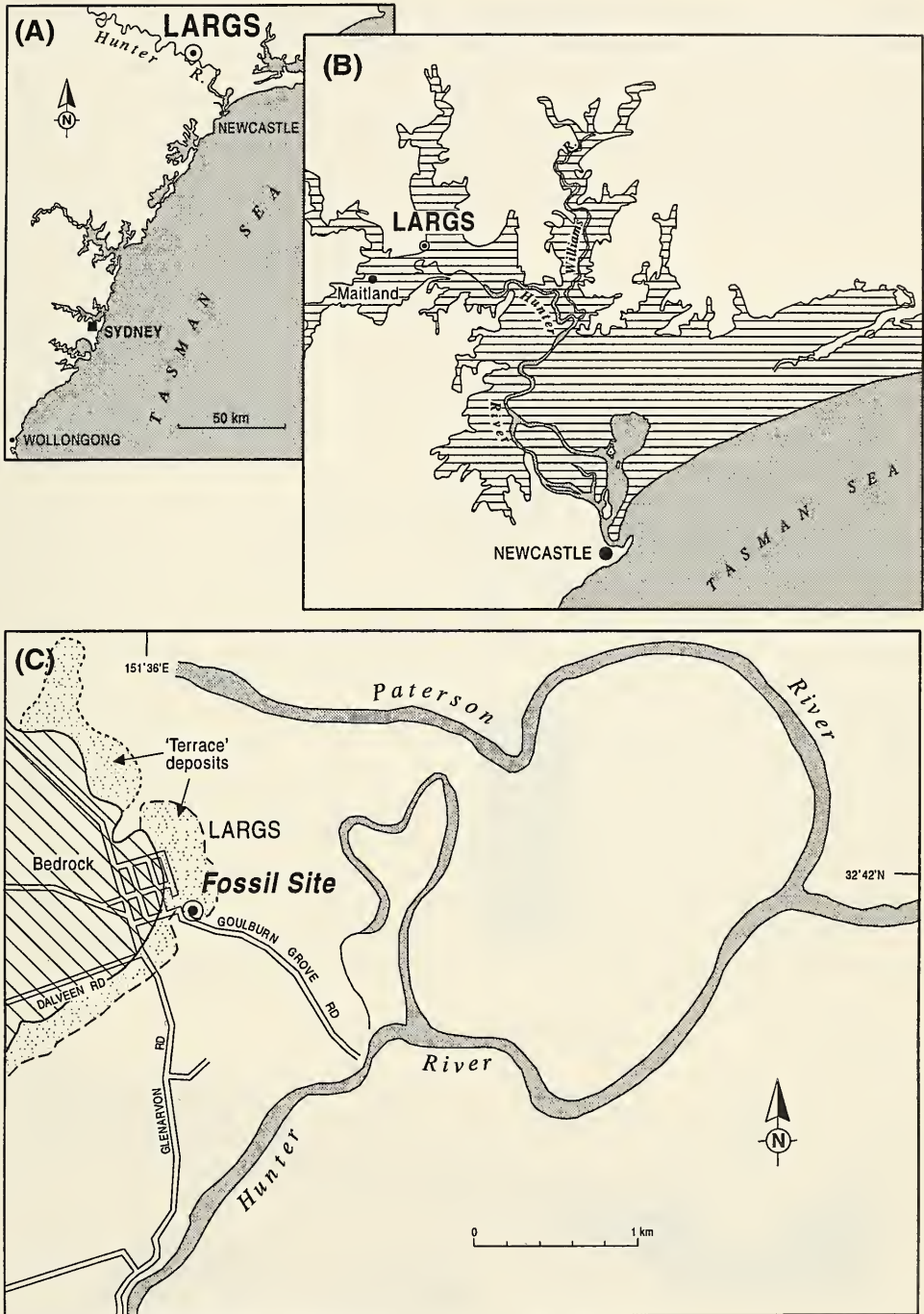
Fig. 1. Location maps showing the general setting of the last interglacial estuarine shell bed at Largs, New South Wales. (a) New South Wales central coast; (b) extent of Late Quaternary deposits in the lower Hunter Valley–Newcastle region, and (c) location of the Largs fossil site (source: Gregory's Newcastle Street Directory, 17th Edition, 1989).
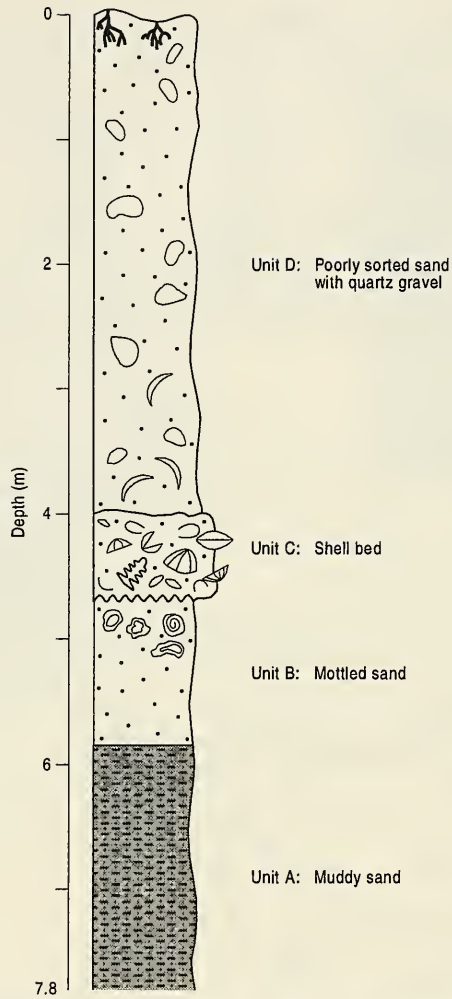
Unit D: Poorly sorted sand
with quartz gravel

Unit C: Shell bed

Unit B: Mottled sand

Unit A: Muddy sand

Depth (m)

Fig. 2. Measured section of the last interglacial estuarine shell bed at Largs, New South Wales.

South

North

Hunter
River

SHELL BED

POSTGLACIAL DEPOSITS
(mainly Holocene)

ESTUARINE MUDS

FLOOD PLAIN SILTS,
ORGANIC-RICH MUDS

PLEISTOCENE DEPOSITS
(mainly last Interglacial)

FLUVIAL SANDS & GRAVELS
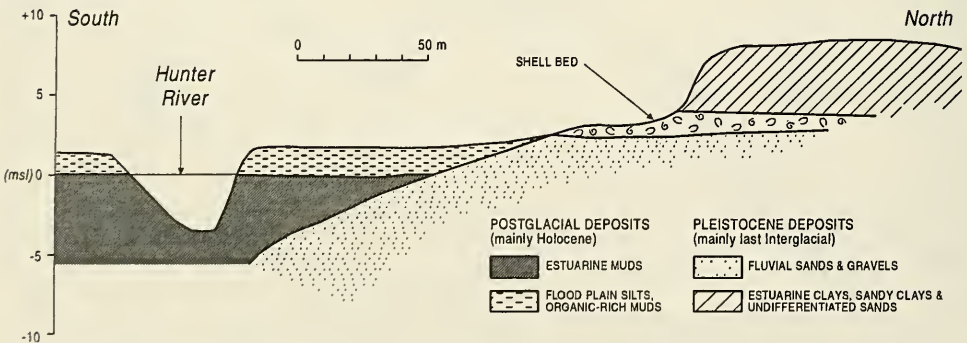
ESTUARINE CLAYS, SANDY CLAYS &
UNDIFFERENTIATED SANDS

Fig. 3. General stratigraphic relationships of the Largs estuarine deposit (after Roy et al. 1995).

minerals. The quartz component consisted of two distinct populations on the basis of surface morphology; a fine-grained, subrounded to rounded quartz population and a sub-angular population of very fine to fine-grained size, reflecting windblown marine and fluvial provenances respectively. The shell-rich horizon is interpreted as an estuarine sand with a strong marine influence. Unit C is overlain by up to 4 m of very poorly sorted, greyish brown sand (unit D; Fig. 2). As the excavation was located on the footslope of a large embankment, only 30 cm of this unit was revealed. Shell fragments account for less than 1% of the sediment in unit C. Large angular pebbles of quartz are common.

Fossil molluscs

The shell-rich unit contains an abundant and diverse assemblage of fossil molluscs. The estuarine bivalve *Anadara trapezia* is by far the dominant species (Table 1). The bivalve *Notospisula trigonella* and the gastropod *Nassarius jonasi* also occur abundantly within the deposit. Twenty-six species of molluscs were recovered from the shell bed, although up to 40 species have previously been identified (Iredale 1951; Thom and Murray-Wallace 1988). For the majority of species, the number of individuals identified at any given depth generally did not exceed twenty (Table 1). Articulated bivalves are common in all species from the fragile *Tellina deltoidalis* and *Notospisula trigonella* to the more robust *A. trapezia*. The molluscs represent a death assemblage with *in situ* reworking of the upper portion of the shell bed resulting from wave action. Separate measurements of the length and height of left and right valves of *A. trapezia* ($n = 310$) and *Trichomya hirsuta* ($n = 40$) revealed a bimodal size distribution indicating that the assemblage had not undergone preferential transportation (Leary 1992).

Collectively the species indicate that deposition occurred in an estuarine environment under conditions of moderate to low wave energy. Equal numbers of left and right valves were noted, providing further evidence for quiet water deposition. The species *Anadara trapezia, Scaeochlamys livida* and *Mimichlamys gloriosa* are common on present-day estuarine mudflats. A maximum water cover of 2 m over the shell-rich unit was inferred (Thom and Murray-Wallace 1988), suggestive of a higher sea level of *ca.* 4 ± 1 m during the last interglacial maximum. Members of the genus *Cymatium* are presently found only in warmer water conditions than prevail around the Newcastle region today, indicating warmer temperatures at the time of deposition of the Largs shell bed. The presence of corals of last interglacial age at Grahamstown (Marshall and Thom 1976) is consistent with warmer ocean surface waters by as much as 2°C during this interval (Murray-Wallace and Belperio 1991).

RESULTS AND DISCUSSION

Radiocarbon dating

Six valves of *A. trapezia* collected from the Largs shell bed yielded an uncorrected radiocarbon age of $34,390 \pm 370$ yr BP (SUA-3008). This result is regarded as a minimum age, reflecting the incorporation during diagenesis of approximately 2% radiocarbon, with a modern activity, which could not be isolated during sample pretreatment. The result is significant, however, for it precludes a Holocene age being assigned to the Largs deposit. A previously reported measurement on a single valve of *A. trapezia* yielded an age of > 37,000 yr BP (Lab. Code: O-1843; Langford-Smith and Thom 1969).

Amino acid racemisation dating

Where possible, well-buried molluscs (i.e. > 1 m) were collected for amino acid racemisation analysis so as to minimize diurnal and seasonal temperature fluctuations,

TABLE 1

Species diversity of fossil molluses in the last interglacial estuarine deposit, Largs, New South Wales. Depth within the shell bed is taken from the upper-bounding surface of Unit C (see Fig. 2).

Species/depth (cm)	0–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65
Anadara trapezia	a	a	a	e	e	e	e	e	e	e	e	e	e
Antetrichonya problematica		a	a	b	b	b	b	b	a	a			
Barnacle sp.								a	a	a			
Bedeva hanleyi					b			b	b				
Cabestana spengleri										a	a	a	
Corbula sp.				b	b	b	b	b	b	b	b	b	b
Cymatium parthenopeum					a		a						
Dentalium sp.													
Herpetopoma aspersa				a	a	a	b	a	a				
Mactridae sp.					a								
Mimachlamys gloriosa				a	a	a	a	a	a	a	a	a	
Monilea lentiginosa				b	b	b	b	b	b	b	b	b	
Nassarius dorsatus				a	a	a	a	a	a				
Nassarius jonasi				d	d	d	d	d	b	b			
Notospisula trigonella				d	d	e	e	d	d	d	d		
Ostrea angasi							a	a	a	a	b	b	
Pecten fumatus							a	a	b	b			
Pinna bicolor						a							
Pitar sp.						b	b	b	b	b	b		
Polinicies conicus				a	a	b	b	b	a	a	a		
Polinicies sordidus				a	a	c	b	b	b	b			
Protholathia contessi					b	b	b	b					
Pyrazus ebeninus							a	a	a				
Scaeochlamys livida				a	a	a	a	a	a	a			
Tellina deltoidalis						a	a	a					
Trichomya hirsuta												b	b

Number of shells: a: 1–9; b: 10–19; c: 20–29; d: 30–39; e: >40

such that longer-term temperature variations associated with climate change were the dominant influence on diagenetic racemisation. To avoid intra-shell amino acid D/L ratio variation, analyses were performed on the hinge region of bivalve molluscs.

Despite some variation related to genus, the extent of racemisation of amino acids in the fossils from the Largs shell bed is representative of values typically obtained for Late Pleistocene fossils from southern Australia (cf., Murray-Wallace et al. 1991; Table 2). The relative extent of racemisation of the different amino acids in each mollusc genus is also in accord with previously published results (Kimber and Milnes 1984). In *Chlamys gloriosa*, the lower extent of racemisation for glutamic acid, leucine and lysine may relate to a genus-effect (Miller and Brigham-Grette 1989). Collectively, however, the extent of racemisation for all amino acids in molluscs from the Largs shell bed is greater than in radiocarbon calibrated specimens of Holocene age from Tom Thumb Lagoon, near Wollongong, New South Wales (Table 2). Comparable extents of racemisation to the specimens of *A. trapezia* from Largs have been noted in *A. trapezia* and the cockle *Katelysia* sp. from other last interglacial coastal deposits in southern Australia (Murray-Wallace et al. 1991; Table 2). An assumption implicit in these correlations is that sites with equivalent current mean annual temperatures are likely to have experienced similar diagenetic temperature histories (Wehmiller 1984).

X-ray diffraction revealed trace quantities of calcite inverted from metastable aragonite in the molluscs dated by radiocarbon and amino acid racemisation. Although this may have influenced the incorporation of younger carbon resulting in the apparent radiocarbon age, the comparable extent of racemisation of amino acids in *A. trapezia* from Largs to those from Port Wakefield in South Australia (Table 2) suggests that the younger carbon introduced to these individuals was not in the form of amino acids. The absence of serine and threonine, two unstable amino acids lost from fossils during early diagenesis supports the integrity of the amino acid racemisation data and indicates that the results are based on indigenous amino acids.

A numeric age for the deposit was derived using the model of apparent parabolic racemisation kinetics of Mitterer and Kriausakul (1989). Accordingly, age is determined by the equation:

$$t = [\ (D/L)_S\ /\ M_c\ ]^2$$

where t is the calculated age, $(D/L)_S$ is the extent of racemisation in a fossil sample of unknown age and M_c is the slope of the line defined as $[\ = (D/L)_{cal}/t^{1/2}]$. In the latter equation, the term D/L_{cal} refers to the extent of racemisation in a fossil of known age, used to determine the slope (M_c) and $t^{1/2}$ is the square root of fossil age of the calibration sample. An age of 103,000 ± 15,500 yr BP was determined based on the extent of leucine racemisation in *Anadara trapezia* from the Largs shell bed. The error term accounts for a 1°C uncertainty in the diagenetic temperature history. Molluscs of interstadial age (40 ka BP; oxygen isotope stage 3) and also dated by amino acid racemisation and radiocarbon from Gulf St Vincent, South Australia (Murray-Wallace et al. 1993) were used to calibrate this numeric age assessment. The calculated age represents a minimum age estimate and we correlate the deposit with the last interglacial maximum, which based on oxygen isotope chronologies from marine cores and uranium-series dating of emergent coral terraces occurred some 134 to 118 ka BP (Martinson et al. 1987; Stein et al. 1993; Zhu et al. 1993). This is the only time in the Late Quaternary, apart from the Holocene, when sea level was sufficiently high to flood the palaeo Hunter Valley. Given that the Largs estuarine shell bed occurs 2 m above present sea level and that a maximum palaeo water cover of 2 m is inferred (Thom and Murray-Wallace 1988), much of the lower Hunter Valley is likely to have been flooded during the last interglacial maximum.

TABLE 2

Extent of amino acid racemisation (total acid hydrolysate) in molluscs from the last interglacial shell bed at Largs, NSW and in Holocene and modern specimens.

Locality	Species and number of analyses	Age	Amino acid D/L ratio*						
			ALA	ASP	LEU	PHE	GLU	PRO	LYS
Quarantine Bay, NSW	*Anadara trapezia* (n=2)	modern	—	0.11	0.03 ±0.001				—
Tom Thumb Lagoon, NSW	*A. trapezia* (n=4)	6750±140 yr cal BP (SUA-3058)	0.32 ±0.001	—	—	0.31 ±0.02	0.18 ±0.004	0.38 ±0.003	—
Largs, NSW	*A. trapezia* (n=4)	~125 ka	0.60 ±0.15	0.55 ±0.07	0.46 ±0.02	0.57 ±0.03	0.36 ±0.02	0.61 ±0.09	0.47 ±0.08
Largs, NSW	*Anetrichomya problematica* (n=2)	~125 ka	0.56 ±0.02	0.70 ±0.06	0.54 ±0.03	0.58 ±0.02	0.40 ±0.02	0.56 ±0.05	0.54 ±0.04
Largs, NSW	*Chlamys gloriosa* (n=1)	~125 ka	0.58	0.69	0.25	0.41	0.26	0.53	0.37
Largs, NSW	*Pinna bicolor* (n=1)	~125 ka	—	0.66	0.45	0.47	0.34	0.70	0.58
Largs, NSW	*Pyrazus ebeninus* (n=1)	~125 ka	0.49	0.69	0.29	0.37	0.47	0.45	—
Largs, NSW	*Trichomya hirsuta* (n=1)	~125 ka	0.64	0.71	0.46	0.56	0.38	0.53	0.53
Port Wakefield, Gulf St. Vincent, SA	*A. trapezia* (n=12)	~125 ka	0.67 ±0.02	0.54 ±0.03	0.51 ±0.02	0.73 ±0.06	0.43 ±0.01	—	—
Port Wakefield, Gulf St. Vincent, SA	*Katelysia rhytiphora* (n=6)	~125 ka	0.72 ±0.03	0.46 ±0.02	0.51 ±0.07	0.67 ±0.04	0.38 ±0.04	—	—

* Amino acids: ALA – alanine; ASP – aspartic acid; LEU – leucine; PHE – phenylalanine; GLU – glutamic acid; PRO – proline and LYS – lysine. ± represents 1 σ standard deviation for between-shell D/L ratio variation.

Few mollusc-rich coastal deposits of last interglacial age are known to crop out or occur in shallow subcrop along the New South Wales coast (Thom et al. 1992). Thus, the Largs estuarine shell bed represents an important reference section for the last interglacial maximum in eastern Australia and from a geochronological perspective, the deposit represents an important benchmark from which to correlate future possible finds of last interglacial strata, and for terrestrial-marine correlations.

CONCLUSIONS

A numeric age of 103 ± 15.5 ka BP is derived for the Largs estuarine shell bed based on the extent of leucine racemisation in *Anadara trapezia* and a racemisation kinetic model of apparent parabolic kinetics. The Largs shell deposit is here correlated with the last interglacial maximum (substage 5*e* of the marine oxygen isotope record) which occurred some 134 to 118 ka BP. The amino acid racemisation data reported for the last interglacial molluscs in this study represents a contribution to the growing body of data that will permit future researchers to correlate Late Quaternary coastal deposits in southeastern Australia.

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